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STEPHANO BROTHERS

Philadelphia 7, Pa.

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Dr. Robert C. Hockett
Tobacco Industry Research Committee
150 E. 42nd Street
New York 17, New York

Dear Dr. Hockett:

Was very pleased to receive your letter of March 19th with your comments on the results of the paramecium test on the five coded samples.

In reference to your suggestion for a titration to evaluate the relative carcinogenicity of the compounds, we have already done this using the activity of 3.4 Benzpyrene as the standard of comparison. We also have another evaluation which we call photodynamic toxicity. The results are as follows:

No.	Identity	Potency as Recorded for Mouse Skin	Photodynamic Toxicity %	Carcinogenic Activity based on 3.4 Benzpyrene
0	3:4 Benzpyrene	Potent	100	100%
1	2-Methyl-3,4-Benzphenanthrene	Unknown	130	43
2	1,2,5,6-Dibenzanthracene	Weak	81	0
3	3-Methyl Cholanthrene	Potent	83	121
4	9-Methyl anthracene	Inactive or very low	79	0
5	3,4,9,10-Dibenzpyrene	Very potent	31	200

The reason I did not originally report the relative activity was that I thought that you just wanted to know if the paramecium test showed activity or not. Further, the scale is based on an assumed linear relationship which I do not know whether or not it is sound assumption. I was quite disturbed that I missed the activity of 1,2,5,6 Dibenzanthracene reporting it as zero when the mouse test shows a weak activity. Anyway, we know that it is quite less than 43% of the activity of 3.4 Benzpyrene.

In searching the literature I have found the following references on the activity of 1,2,5,6 Dibenzanthracene.

Wolmay Chemical Abstracts 1940 P. 4469
"Has a proliferative effect on paramecium at an optimum concentration of 1 in $2x10^5$"

Further Mottram Cancer Research Vol. 1, P. 313 said
"Leads to production of abnormal forms of paramecia even in the dark"

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and further Mottram & Doniach, Nature 140 P. 933 said
"Does not affect the motility of paramecium in the dark but at a concentration of 1 in 10^4 it is rapidly lethal on irradiation with light of wave length of 350-410 Mμ or with sunlight has similar activity on the infusorium coleps"

From the above two references giving concentrations of 1,2,5,6-Dibenzanthracene these reactions discussed occur at concentrations of 20-200 times stronger than those used in my tests.

I chose the level of concentration for the test of these unknowns on the basis of the level I used in calibrating the 3:4 benzpyrene curve for my experimental set-up and the curve gives the best response at a concentration of 1 in 10^6 to 1 in 10^8. It would seem that this test of an unknown should be conducted as I dealt with it in the case of the five unknowns to separate the strong from the weak but when the first test indicates a weak or zero activity further tests should be run in the 1 part in 10^3 to 1 part in 10^5 level.

This should enable us to escape overlooking weak activity and evaluate same in terms of the activity of 3:4 Benzpyrene. On this basis I roughly estimate the activity of 1,2,5,6-Dibenzanthracene to lie between 0.5%-5.0% of the carcinogenic activity of 3:4 Benzpyrene.

In reference to further evaluation of the test, as I told you during our recent meeting I am very busy with work in the nature of cigar smoke so it would be very good if you could interest some academic institution to further explore the method; as to response of all known P.A.H.C. of carcinogenic nature; to the paramecium test.

Further it would be very good if the Scientific Advisory Board of the T.I.R.C. chose to have normal cigarette smoke as I define it in my paper checked against abnormal cigarette smoke on mouse skin tests being sure that the smoke used is normal or abnormal in the basis of the M.E.T. rise puff by puff. This would really answer the question under consideration on the basis of methods accepted today.

Further statistical studies of the population on samples properly chosen from the statistical viewpoint would confirm or deny my proposition that the average smoker does not exceed an M.E.T. rise of 5.1°C.

M.E.T. Rise Studies on persons afflicted with lung cancer would be of great interest.

These tests and others that the Scientific Advisory Board of the T.I.R.C. might think of, such as tissue culture test, would enable us to challenge on a firm basis the Wynder thesis using his own tools. With best regards, I am

Very sincerely yours,
STEPHANO BROTHERS

/s/ C. S. Stephano

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